however, is given by the oligonucleotide GpGpGpApGpApCp (No. 425) derived from complete digestion of the RNA by pancreatic ribonuclease. It is the only oligonucleotide from Table 1 not already accounted for in the partial-digestion fragments obtained.

Since it is the only pancreatic ribonuclease product ending in . . . GpApCp, it must overlap with the oligonucleotide ApCpCpGp which is the only T1 ribonuclease digestion product beginning in ApCp . . . and is present at the far left of the 3′-end region of the RNA. In summary, the results shown in Fig. 3 indicate the complete linear sequence of KB cell 5*S* RNA.

The presence of regions in the molecule that show marked and reproducible variability in susceptibility to digestion by T1 ribonuclease, as noted above, suggests that the molecule has a specific secondary structure. If, in addition to classic Watson-Crick base-pairing of nucleotides, one proposes occasional Gp-to-Up base-pairing, as assumed in the cloverleaf model for tRNA (*7, 8*), it is possible to construct different models of the possible secondary structure of KB cell 5*S* RNA. The molecule contains two sets of complementary sequences, nine nucleotides in length. Residues 1 through 9 (Fig. 3) can form base pairs with residues 118 through 110, thus joining the 5′-end to the 3′-end of the molecule as in the cloverleaf model for tRNA (*7, 8*); and residues 27 through 35 can form base pairs with residues 67 through 59. There are a number of additional complementary sequences containing from 3 to 6 nucleotides. They can be arranged in different patterns. Selection of a final model of the secondary structure of KB cell 5*S* RNA will depend on the detailed comparison of its sequence to that of 5*S* RNA in other cell species, such as that of *E. coli* 5*S* RNA (*12*). In *E. coli* 5*S* RNA there is also the possibility for base pairing of the 5′-end of the RNA with its 3′-end for a length of 10 nucleotides

In KB cell 5*S* RNA there are two sequences, one of seven nucleotides and another of six nucleotides, which occur twice in the molecule: the sequence GpApUpCpUpCpGp (residues 31 to 37 and 41 to 47) and the sequence GpCpCpUpGpGp (residues 66 to 71 and 93 to 98). In *E. coli* 5*S* RNA there are sequences of ten and eight nucleotides which also occur twice (*12*). However, in *E. coli* 5*S* RNA these sequences are repeated in different halves of the molecule, while in 5*S* RNA from KB cell they are repeated in the same half of the sequence.

In most models of the secondary structure of KB cell 5*S* RNA, the sequences GpApApUp (residues 99 to 102 and UpGpApApCp (residues 20 to 24) are not involved in base pairing. These sequences are complementary in an "antiparallel" fashion to four or five bases in the pentanucleotide sequence GpTpψpCpCp which is common to most tRNA's and is tentalively located in the loop of the limb of the molecule nearest its 3′-end (*7, 13*). This association may be fortuitous or it may be related to the function of 5*S* RNA in the ribosome. It is of interest that *E. coli* 5*S* RNA contains the sequences GpApApCp (residues 107 to 110) and CpGpApApCp (residues 43 to 47) which are also complementary to four or five bases in the pentanucleotide sequence GpTpψpCpGp of tRNA (*12*).

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References and Notes

1. R. Rosset, R. Monier, J. Julien, *Bull. Soc. Chim. Biol.* **46**, 87 (1964).
2. J. Marcot-Queiroz, J. Julien, R. Rosset, R. Monier, *ibid.* **47**, 183 (1965); D. G. Comb, N. Sarkar, J. DeVallet, C. J. Pinzino, *J. Mol. Biol.* **12**, 509 (1965); F. Galibert, C. J. Larsen, J. C. Lelong, M. Boiron, *Nature* **207**, 1039 (1965).
3. F. Sanger, G. G. Brownlee, B. G. Barrell, *J. Mol. Biol.* **13**, 373 (1965).
4. G. G. Brownlee and F. Sanger, *ibid.* **23**, 337 (1967).
5. B. G. Forget and S. M. Weissman, *Nature* **213**, 878 (1967).
6. J. Hindley, personal communication.
7. R. W. Holley, J. Apgar, G. A. Everett, J. T. Madison, M. Marquisee, S. H. Merrill, J R. Penswick, A. Zamir, *Science* **147**, 1462 (1965).
8. J. T. Madison, G. A. Everett, H. Kung, *ibid.* **153**, 531 (1966).
9. H. G. Zachau, D. Dutting, H. Feldmann, *Z. Physiol. Chem.* **347**, 212 (1966).
10. U. L. RajBhandary, S. H. Chang, A. Stuart, R. D. Faulkner, R. M. Hoskinson, H. G. Khorana, *Proc. Nat. Acad. Sci. U.S.* **57**, 751 (1967).
11. Abbreviations used: tRNA, transfer ribonucleic acid; PME, alkaline phosphomonoesterase; DEAE, diethylaminoethyl; tris-HCl, tris (hydroxymethyl)aminomethane hydrochloride; T, ribothymidine; Ψ, pseudouridine; A, adenosine; C, cytidine; G, guanosine; U, uridine; the subscript OH is used to indicate the presence of a 3′-hydroxyl group; p, on the left of A, C, and the like, indicates a 5′-phosphate; and on the right, a 3′-phosphate. In Fig. 3 and Table 1, hyphens are used instead of p.
12. G. G. Brownlee, F. Sanger, B. G. Barrell, *Nature* **215**, 735 (1967).
13. A. Zamir, R. W. Holley, M. Marquisee, *J. Biol. Chem.* **240**, 1267 (1965).
14. Supported in part by grant ACS-E456 of the American Cancer Society. Miss Carol Hybner and Mrs. Iris Morton contributed technical assistance.
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Photoreactivation in vivo of Pyrimidine Dimers in Paramecium DNA

Abstract. *Cells of* Paramecium aurelia *labeled with tritiated thymidine were irradiated with ultraviolet light and then were either exposed to photoreactivating light or kept in the dark as controls. In the controls, the level of thymine-containing pyrimidine dimers did not change, but in cells exposed to photoreactivating light such dimers were destroyed. This is the first demonstration in a eukaryote of in vivo photoreactivation of thymine-containing pyrimidine dimers.*

Ultraviolet light (2200 to 3000 Å) causes both death and mutation in microorganisms and higher organisms. A large body of evidence indicates that cyclobutane-type pyrimidine dimers in DNA of irradiated organisms are important in the lethal and mutagenic effects of ultraviolet light (*1, 2*). The most convenient method for evaluating the biological role of these dimers is by means of photoreactivation—reversal of the ultraviolet-induced damage by irradiation with light of longer wavelengths. The photoreactivating ability of an organism may be observed in two ways: (i) biological photoreactivation, and (ii) the destruction of ultraviolet-induced pyrimidine dimers in vitro (*3*) and in vivo (*4*). In microorganisms the concurrent presence or absence of these two phenomena permits evaluation of the role of dimers in the lethal and mutagenic effects of ultraviolet light; however, such an evaluation has not been possible for organisms higher than bacteria. Although dimers have been found in the DNA of eukaryotes as a result of irradiation with ultraviolet light (*5*), there have been no demonstrations of in vivo destruction of dimers in eukaryotes. We report the first such case: the in vivo photoreactivation of thymine-containing pyrimidine dimers in the DNA of *Paramecium aurelia.*

The DNA of paramecia (syngen 7, stock 57) was labeled specifically (*6*) by letting the animals feed overnight on cells of *Escherichia coli* 15T$^-$ that had been grown in medium containing ^{3}H-methyl thymidine with high specific activity. The paramecia were irradiated in Dryl's (*7*) solution, which is transparent to ultraviolet light. A germicidal lamp, emitting mainly at 2537 Å, supplied the ultraviolet light exposure of 4000 erg mm^{-2} at a rate of 55 ergs

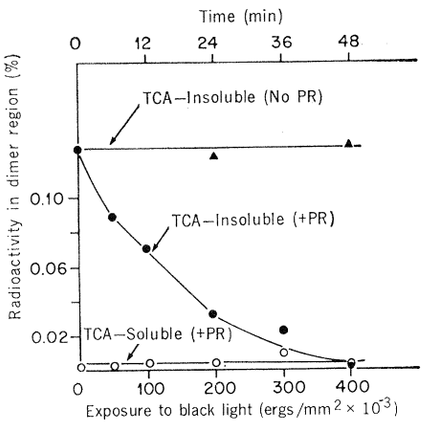

Fig. 1. The thymine-containing dimers in the TCA-insoluble (●) and TCA-soluble (○) fractions of paramecia irradiated with ultraviolet light after several exposures to photoreactivating light (PR). As a control, irradiated animals were kept in the dark after ultraviolet irradiation for the same periods of time as were used for photoreactivation. The dimers in the TCA-insoluble (▲) fractions were determined for the controls.

mm^{-2} sec^{-1}, as estimated from Jagger meter (*8*) readings. After this exposure, cell survival at 48 hours was 100 percent.

After irradiation the cells were divided into three groups. The first group was killed immediately by the addition of ice-cold, 5 percent trichloroacetic acid (TCA); the second was exposed to photoreactivating light (PR) from a General Electric BLB black-light bulb at a rate of 8000 ergs mm^{-2} sec^{-1}. A glass plate between the animals and the black-light bulb prevented transmission of wavelengths below about 3000 Å. The third group was placed in a lightproof chamber and killed at the same time as the corresponding sample exposed to PR, the cells being centrifuged from Dryl's solution and killed by the addition of TCA. Three ether extractions were used to remove the TCA from the TCA-soluble fractions. Calf thymus DNA was added to aid in chromatographic identification, and the samples were hydrolyzed for 30 minutes in 97 percent formic acid at 175°C. The hydrolysates were analyzed for radioactivity in thymine and thymine-containing dimers by two-dimensional paper chromatography, elution, and counting in a dioxane-based system (*4*) in a scintillation counter. The photoproducts were identified as dimers by their chromatographic mobility and by the kinetics and products of their monomerization at 2390 Å (*9*). It will be shown elsewhere (*10*) that the photoproducts measured were indeed dimers from *Paramecium* DNA, even though bacteria were used to label the paramecia.

Figure 1 shows the results of a typical experiment designed to determine the fate of dimers in cells irradiated with ultraviolet light that were subsequently exposed to PR or kept in the dark. In the dark the dimer content did not change; in the light, dimers disappeared from the TCA-insoluble fraction and did not appear in the TCA-soluble fraction—nor did they appear in the medium, because only 0.04 percent of the total radioactivity was released into the medium during the experiment. Even if all the material released from the cells were dimers, it would not be great enough to account for the disappearance of dimers in the light. These results strongly indicate that, in the presence of PR, dimers in *Paramecium* DNA are monomerized in vivo.

Mutational damage produced by similar doses of ultraviolet light are also reactivated (*11*) by exposures to photoreactivating light comparable to those used in the present experiments (*12*). However, evaluation of the biological role of pyrimidine dimers in mutagenesis due to exposure to ultraviolet light is not yet possible, because the dose-response relation for induction and photoreactivation of mutation in *Paramecium* by ultraviolet light has not been completely worked out. Nevertheless, the destruction of dimers in vivo by PR suggests that biological photoreactivation of mutation in *Paramecium* results from the monomerization of pyrimidine dimers, and that the induction of the mutations by ultraviolet light results from the formation of pyrimidine dimers.

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References and Notes

1. See reviews by A. Wacker, *Progr. Nucleic Acid Res.* **1**, 369 (1963); K. C. Smith, in *Photophysiology*, A. C. Giese, Ed. (Academic Press, New York, 1964), vol. 2, p. 329; R. B. Setlow, in *Mammalian Cytogenetics and Related Problems in Radiobiology*, C. Pavan, C. Chagas, D. Frota-Pessoa, and L. R. Caldas, Eds. (Pergamon, Oxford, 1964), p. 291.
2. E. M. Witkin, N. A. Sicurella, G. M. Bennett, *Proc. Nat. Acad. Sci. U.S.* **50**, 1055 (1963).
3. C. S. Rupert, *J. Gen. Physiol.* **43**, 573 (1960); D. L. Wulff and C. S. Rupert, *Biochem. Biophys. Res. Commun.* **7**, 237 (1962); see review by J. K. Setlow, in *Current Topics in Radiation Research*, M. Ebert and A. Howard, Eds. (North-Holland, Amsterdam, 1966), vol. 2, p. 195.
4. R. B. Setlow, P. A. Swenson, W. L. Carrier, *Science* **142**, 1464 (1963).
5. J. Trosko, E. H. Y. Chu, W. L. Carrier, *Radiation Res.* **24**, 667 (1965); G. L. Whitson, A. A. Francis, W. L. Carrier, *J. Protozool.* **14** (suppl.), 8 (1967).
6. J. D. Berger and R. F. Kimball, *ibid.* **11**, 534 (1964).
7. S. Dryl, *ibid.* **6** (suppl.), 96 (1959).
8. J. Jagger, *Radiation Res.* **14**, 394 (1961).
9. K. C. Smith, *Photochem. Photobiol.* **2**, 503 (1963); R. B. Setlow and W. L. Carrier, *J. Mol. Biol.* **17**, 237 (1966).
10. B. M. Sutherland, W. L. Carrier, R. B. Setlow, in preparation.
11. R. F. Kimball and N. Gaither, *J. Cell. Comp. Physiol.* **37**, 211 (1951).
12. R. F. Kimball, unpublished.
13. Sponsored by the U. S. Atomic Energy Commision under contract with the Union Carbide Corporation.
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Frog Skeletal Muscle Fibers: Changes in Electrical Properties after Disruption of Transverse Tubular System

Abstract. *In muscle fibers which have been exposed for 1 hour to a Ringer solution containing 400 millimolar glycerol and then returned to plain Ringer solution, the transverse tubular system is disrupted. At the same time the membrane capacitance is markedly reduced and hyperpolarizing current pulses no longer produce a slow, progressive increase in potential (creep). The large capacitance of muscle and the phenomenon of "creep" must both depend on an intact transverse tubular system.*

The values of the membrane capacitance of nerve and muscle are strikingly different, being of the order of 1 μfarad/cm^2 in nerve (*1*) and 4 to 8 μfarad/cm^2 in skeletal muscle of frogs (*2*, *3*). The higher membrane capacitance of muscle fibers has been attributed to the presence of two components, the surface membrane and the membrane of the transverse tubular system (*3*, *4*). From measurements of impedance in frog skeletal muscle (*3*) the surface membrane appears to have a capacitance of 2.6 μfarad/cm^2 and